

## OBSERVATION OF SPERMATOPHORE TRANSFER IN *STAVSOLUS JAPONICUS* (PLECOPTERA: PERLODIDAE) IN JAPAN<sup>1</sup>

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**ABSTRACT:** Behavior patterns associated with spermatophore transfer have been studied in several species of insects, but in stoneflies, even the existence of a spermatophore has been uncertain. This study aimed to confirm the existence of spermatophores in *Stavsolus japonicus* (Plecoptera: Perlodidae) and to determine the manner of spermatophore transfer. The male bent its body into an S-shape and inserted its abdominal caudal tip between the sternites and the subgenital plate of the female to open the female's subgenital plate. After approximately one hour, the male started to stroke the female's eighth and ninth genital segments with its epiproct. Next, the aedeagus was extruded between the male's eighth and ninth sternites and a spermatophore-like mass was extruded from the aedeagus. The mass contained sperm and was thus considered to be a spermatophore. The spermatophore was placed directly under the female's subgenital plate by the aedeagus and the male then patted the spermatophore with his cerci.

**KEY WORDS:** stonefly, spermatophore, copulation, cerci, subgenital plate, Plecoptera, perlodidae, *Stavsolus*, Japan

Sperm transfer of insects is accomplished in several ways and the methods of sperm transfer from males to females can be divided into two types: (1) direct transfer of sperm into the female duct, and (2) transfer to the female as a spermatophore. The first method, direct sperm transfer, is common in the Neoptera. The second method, spermatophore transfer, is an indirect mode of sperm transfer, and is regarded as a primitive but common method of insemination (Davey, 1960; Proctor, 1998). In the primitive hexapods, such as the Collembola, Diplura and Thysanura, spermatophore transfer is a simple process: the male deposits the spermatophore on a substrate that is subsequently recovered by the female (Schaller, 1971). In other group of insects, the males convey the spermatophore directly to the female. The transferred spermatophore remains outside the female genital duct in most cases, and in ensiferan Orthoptera only the neck of the spermatophore penetrates the female genital duct. In some Megaloptera, female eat the spermatophore, during which time the sperm migrates to the female (Hayashi 1999). Although in Blattodea the transferred spermatophore also remains outside the female genital duct, it is protected by the female's enlarged subgenital plate (Khalifa, 1950).

Sperm transfer in Plecoptera was previously described by Brink (1956). He divided plecopterans into two categories: (1) the species with a ventral penis or penial organ functioning as a direct sperm conveyor through copulation with internal deposition, and (2) the species without a ventral penis, aedeagus, or

<sup>1</sup> Received on April 23, 2007. Accepted on May 21, 2007.

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penial organ, the conveyance of the sperm is undertaken by the epiproct. Brink (1956) also indicated that all the males of Plecoptera pass the sperm directly into the female duct rather than producing spermatophores. In 1969, Stewart et al., described copulation behavior in *Perlesta placida* (Hagen) (Perlidae). In these insects, sperm insertion is accomplished by hooking the paraprocts and the male then seals off the female genitalia using the expanded copulatory organ, indicating a direct sperm transfer. However, Stewart and Stark (1977) reported that a mucoid sperm mass is deposited in *Hydroperla crosbyi* (Needham and Claassen) (Perlodidae), and suggested three possible methods of sperm transfer in Plecoptera. Since this supposition was reported in 1997, no subsequent observations about spermatophore have been published. The purposes of this study are to confirm the existence of a spermatophore in stoneflies and to determine the method of spermatophore transfer in *Stavsolus japonicus* (Okamoto) (Plecoptera: Perlodidae).

## METHODS

From March 31 to April 12, 2005, newly emerged adults of *S. japonicus* males ( $n = 14$ ) and females ( $N = 16$ ) were collected in the morning from the Shigo River at Aritoshi, Nara Prefecture, Japan ( $34^{\circ}23'N$ ,  $136^{\circ}00'E$ ). All individuals emerged the morning they were collected. The stoneflies were brought to the laboratory and kept individually in plastic vials (diameter x height: 3 x 6.5 cm) at  $15 \pm 1^{\circ}C$ . They were fed diluted honey (honey: water, 1:10) almost every day using soaked cotton.

Two weeks after the date of collection, a female and a male were put together in a plastic vial (diameter x height: 3 x 6.5 cm) at 11:00 once every two or three days for copulation. Copulation behavior and duration were recorded. If they did not copulate, pairs were separated at 16:00. Females that copulate once were not used for other experiments. Females that did not copulated were used in later copulation experiments with other males. Males, however, were allowed to copulate during their lifetime. Ten of sixteen females copulate. Dead individuals were preserved in 80% ethanol.

After copulation, a spermatophore-like mass transferred to females was carefully removed either immediately after copulation, or at 15, 30, or 45 minutes after completion of copulation (the masses were removed from two females for each interval) using fine forceps. The spermatophore-like masses were placed on a glass microscope slide with an eosin stain. After a cover slip was placed on it, the mass was examined microscopically for sperm. The spermatophore-like masses on two remaining females were left undisturbed and were monitored to determine the time required for the spermatophore-like mass to disappear from sight. All the females were kept at  $15 \pm 1^{\circ}C$  in plastic vials and were observed to determine whether or not oviposition occurred.

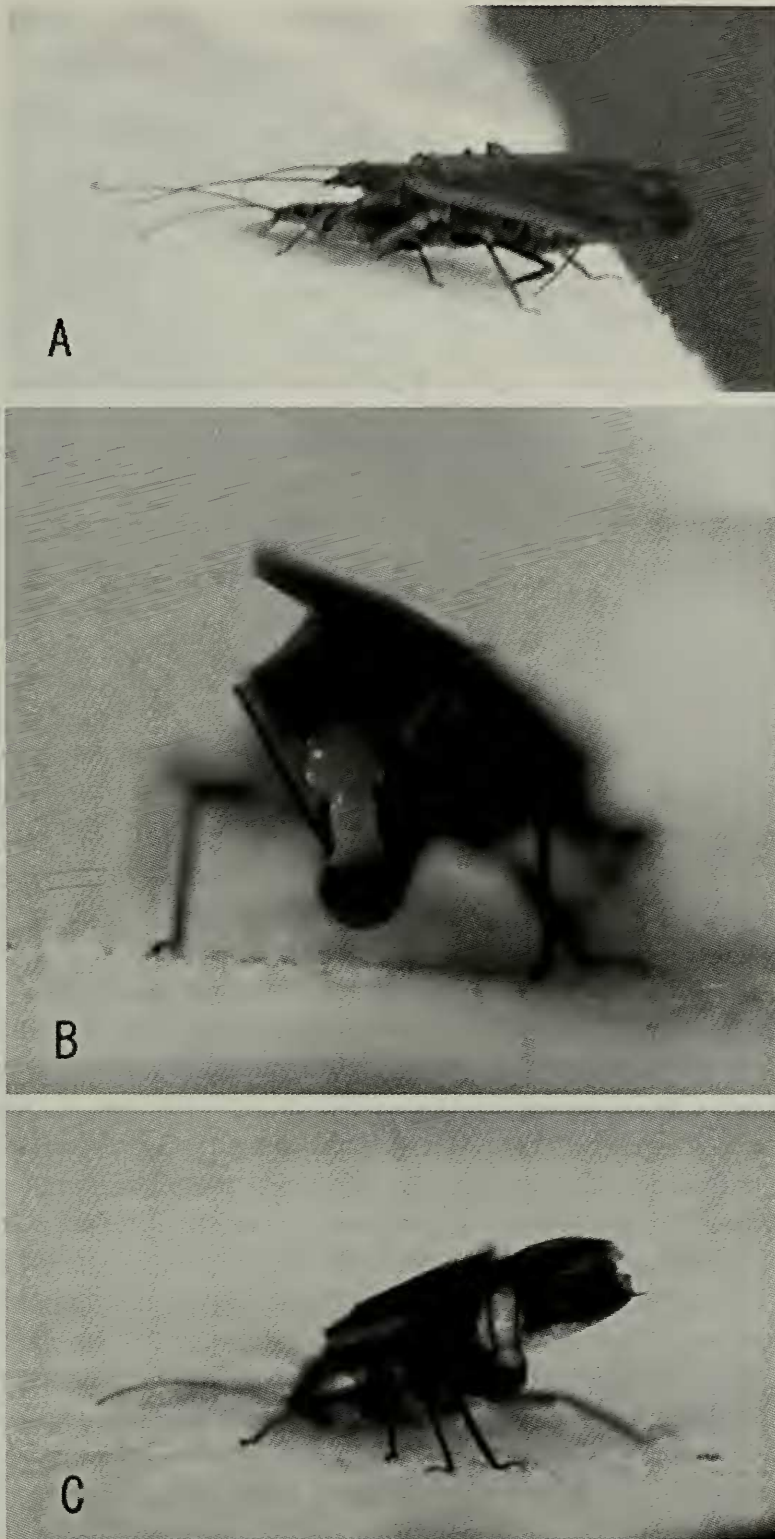


Fig. 1. Copulation behavior in *S. japonicus*. A. Before the spermatophore transfer. B. Spermatophore transfer. C. After the spermatophore transfer.

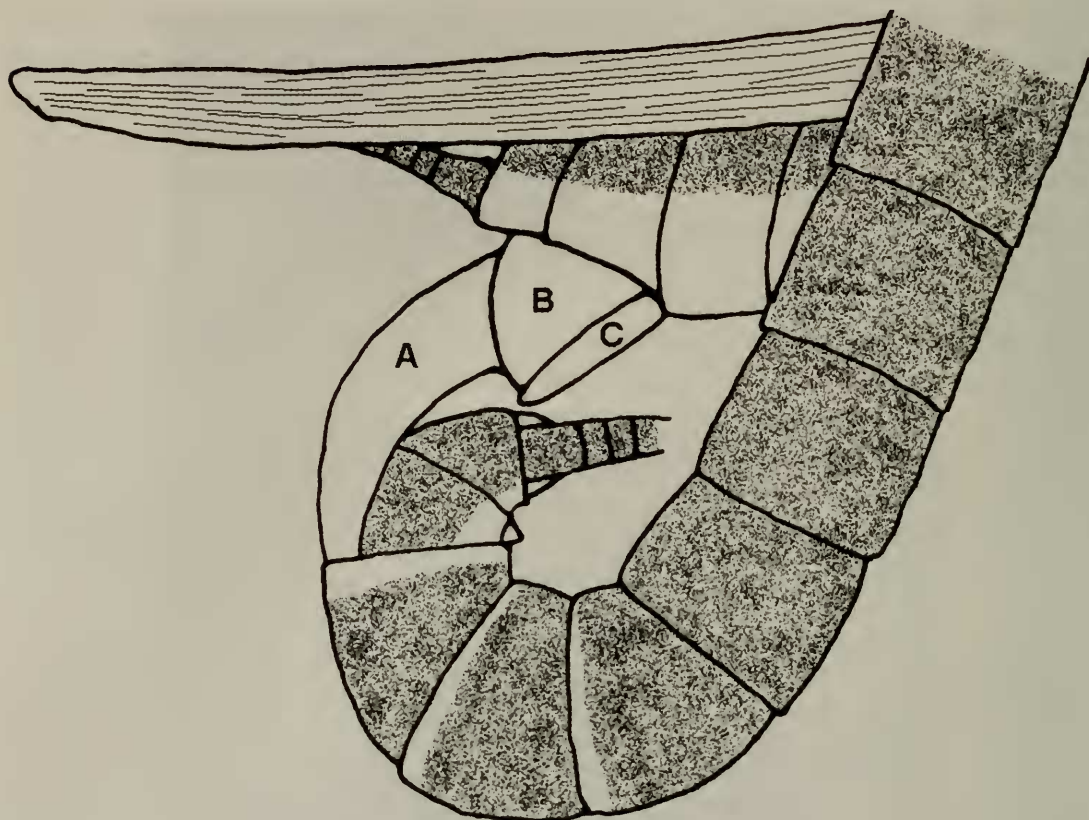


Fig. 2. Diagram of spermatophore transfer. A. Aedeagus, B. Spermatophore. C. Subgenital plate.

## RESULTS

When a male found a female of the stonefly *Stavsolus japonicus*, he approached and tried to mount her back without drumming. When mounting was successful, the male bent his body into an S-shape and attempted copulation (Fig. 1A). Females of this species have a large subgenital plate on the eighth sternite and the edge of the plate extends to the middle of the tenth sternite. The male opened the female's subgenital plate with his abdominal caudal tip and inserted his genitalia or abdominal caudal parts between the female's eighth and ninth sternites and the subgenital plate. The male then thrust his abdominal caudal parts into the female as continuing to gently wave his abdomen. Once the male and female genitalia were linked, the female did not move around and the male did not pull out his abdomen. If, however, the connection was not complete, the male sometimes retracted his abdomen from the female's genitalia. In this case, the female sometimes began to move around, which is a reason to prolong the copulation period. This stage of copulation lasted between 40 and 90 minutes. Next, the male stopped pushing his abdominal caudal tip and stroke (ca. twice / s) the female's eighth and ninth genital segments under the subgenital plate using his epiproct for approximately two minutes. After that, the male's aedeagus was extruded between the eighth and the ninth sternites and was extended nearer to the female space between sternites and subgenital plate, and then a white and

translucent mass that looked like a spermatophore was extruded from the aedeagus. The spermatophore-like mass was inserted directly under the female's subgenital plate by male's aedeagus (Fig. 1B, Fig. 2). After the insertion of the mass-like spermatophore, the male patted the spermatophore-like mass by moving his cerci up and down (Fig. 1C). Even if the female began to move around, the male continued this patting for approximately five minutes and then dismounted from the female. The male did not show any interest in the female after copulation. Total time for copulation ranged from 55 to 105 minutes ( $74 \text{ min} \pm 19 \text{ min}$ , mean  $\pm$  S.D.;  $n = 10$ ).

The spermatophore-like mass was sticky and contained sperm in a gelatinous mass. Thus, this mass was, in fact, a spermatophore. The transferred spermatophore was protected by the female's comparatively large subgenital plate and was absorbed in approximately two hours. When the spermatophore was artificially removed immediately after copulation, the female did not oviposit. However, when the spermatophore was removed 15, 30 or 45 minutes after completion of copulation, the females did oviposit, despite the fact that the removed spermatophore contained sperm.

## DISCUSSION

Sperm of *S. japonicus*, Perlodid stonefly, was transferred as a spermatophore by male's aedeagus and the spermatophore was directly inserted under the female's subgenital plate. Male's aedeagus was not directly inserted into the female genital organ in this study. This shows that *S. japonicus* does not fit into either of the categories defined by Brink (1956). The fact of external deposition of spermatophore observed in this study was similar to that observed in *H. crosbyi*, where the spermatophore-like mucoid sperm mass is deposited directly under the female's subgenital plate (Stewart and Stark, 1977). Although Brink (1956) divided the Plecoptera into two categories, he mentioned the "loose fix" of copulation, which is due to the lack of any fixing hooks in the genera *Diura* and *Perlodes* (Perlodidae) that were categorized as having direct sperm transfer. Based on the supposition of three different types of sperm transfer in Plecoptera by Stewart and Stark (1977), the mention of "loose fix" of copulation by Brink (1956), and the result of this study, some perlodids might transfer the sperms as spermatophores. Although the type of sperm conveyed in *S. japonicus* of this study is similar to that seen in *H. crosbyi* (Stewart and Stark, 1977), it has not been previously reported in any other plecopteran group. It will be very interesting when this method is restricted only in Perlodidae of Perloidea. Surdick (1985) reported the absorption of liquid sperm in *Sweltsa* (Chloroperlidae), although Yoshimura et al. (2003) observed a spermatophore-like object after copulation in *Sweltsa* sp. Further studies are needed to confirm the type of sperm conveyance in Perloidea.

A reduced epiproctal apparatus is widespread among Perloidea, but the role of the epiproct in Perloidea mating is not well understood (Zwick, 2000). In this study, the male stroked the female's eighth and ninth genital segments under the subgenital plate with the epiproct before extruding the spermatophore. This behavior may serve to clean and stimulate the female's genital segment. Spermatophore was extruded just after stroking; therefore, this behavior would be indispensable in their courtship copulation and the epiproct might probably play an important role for sperm conveyance.

The male of *H. crosbyi* engages in a side to side brushing or a delicate tapping with his cerci on the female cerci. Then, the female begins a rhythmic telescoping action of her abdominal segments behind the genital opening (Stewart and Stark, 1977). In contrast, the behavior of *S. japonicus* after deposition of spermatophore was different. The males of *S. japonicus* patted the spermatophores up and down with their cerci for a few minutes before dismounting, indicating that the cerci may play a certain role in copulatory courtship.

In *Calliptamus* (Orthoptera), the shape of cerci is different between males and females, and in *Idioembia* (Embioptera) the cerci of males are asymmetric. Some insects use the cerci for holding the female during copulation. Besides, the cerci seem to have some contact chemoreceptors and olfactory receptors (Merritt and Rice, 1984). Therefore, males may receive some stimulation from female cerci in *H. crosbyi* and from the spermatophore in *S. japonicus* of this study. The females of *S. japonicus* did not show obvious abdominal telescoping action in this study; thus, male's patting on the spermatophore with his cerci might lead the male to know the various conditions about spermatophore by receiving some signals and lead the female to stimulate the sperm absorption.

In Blattodea, female's long subgenital plate protects the spermatophore and males do not display patting behavior. Tapping behavior on the spermatophore in Plecoptera might be used to ensure the spermatophore not to drop off of the female after spermatophore transfer. Female crickets sometimes attempt to remove the inserted spermatophores. And the males of *Teleogryllus natalensis* and *Gryllus bimaculatus* have to guard the females against this removal (Sakaluk, 1991; Hockham and Vahed, 1997; Wynn and Vahed, 2004). On the other hand, the males of *Cycloptiloides canariensis* do not display guarding behavior. Although the females detach the spermatophores about 30 seconds after the transfer, sperm in the spermatophores are emptied within 35 seconds, so the postcopulatory guarding may be unnecessary (Dambach and Beck, 1990). Postcopulatory guarding was not observed in *S. japonicus* in this study. Sperm transfer from the spermatophore would start within fifteen minutes from the completion of copulation, since oviposition occurred when the spermatophore was removed fifteen or more minutes after completion of copulation. By using the male's cerci for pushing the spermatophore further onto the female's comparatively larger subgenital plate, it might be difficult for the female to drop the spermatophore and be unnecessary for males to guard the females after copulation.

In the cricket *Gryllus bimaculatus*, bodily movement ceases during copulation except for the antennae and cerci before the spermatophore is inserted into the female genitalia. This immobility appears to be due to a vibratory stimulus from the male's thrusting movements underneath the female (Sakai and Kumashiro, 2004) and may enable safe transfer of the spermatophore. Copulation lasts only a few minutes in this cricket (Sakai and Kumashiro, 2004), so it is presumably easy for the male to induce paralysis in the female by abdominal vibrations for short periods. In this study, the copulation period of *S. japonicus* varied from 55 minutes to 1 hour 45 minutes, and the longer copulation period was the result of a longer period of thrusting and waving of the male's abdomen. In order to transfer the spermatophore safely, the male needs the female to be immobile. The thrusting and

abdominal waving might be used by the male to induce paralysis in the female, in a fashion similar to that observed in the cricket. Stroking the membranous segment with his epiproct may function to confirm that the female is paralyzed before the spermatophore is inserted. Alternatively, the male may perform this copulatory behavior to clean the female genital organ and to make it easier for sperm from the spermatophore to be absorbed. In the cricket, the male's first act during copulation is to stimulate some receptors, which in turn elicit another act and so on (Sakai and Kumashiro, 2004). A similar chain reaction system might also exist in stoneflies. Further examination of the connected genitalia could clarify the significance of these copulatory behaviors in *S. japonicus*, and would help further advance our knowledge of sperm conveyance in Perloidea and our understanding of the copulation strategies in Plecoptera and Insecta.

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